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Title5-11
DIFLUORO ANTIVIRALS AND INTERMEDIATE THEREFORCross-Reference to Related Application

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This is a continuation-in-part of my copending application Serial No. 473,883, filed March 10, 1983.

Background of the InventionField of the Invention

10
15 This invention belongs to the field of pharmaceutical chemistry, and provides a new difluoro carbohydrate and new antiviral nucleosides prepared by coupling the new carbohydrate with appropriate bases.

State of the Art

20
25 It has been known for some time that antiviral drugs can be found among the general family of nucleosides. For example, 5-(2-bromovinyl)-2'-deoxyuridine is known to be a potent agent against herpes virus. DeClercq et al., Proc. Natl. Acad. Sci. USA 76, 2947-51 (1979). Watanabe et al. have described a number of nucleosides formed by coupling 2-fluoro-2-deoxyarabinofuranose with bases of the cytosine and thymine groups; 5-iodocytosine was their most preferred
30 base. J. Med. Chem. 22, 21-24 (1979), and U.S. Patent 4,211,773.

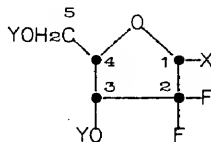
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15 A compound which can be described as an acyclic nucleoside, 9-(2-hydroxyethoxymethyl)guanine, is a potent antiviral agent, especially useful against herpes viruses, and is the subject of a symposium in a special issue of American Journal of Medicine, July 1982.

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10 Fluorinated carbohydrates have been studied before. A survey of the subject by Penglis is in Advances in Carbohydrate Chemistry and Biochemistry 38, 195-285 (1981). A 2,2-difluorohexose was described by Adamson et al., Carbohydrate Research 18, 345-47 (1971). Wright and Taylor, Carbohydrate Research 6, 347-54 (1968), taught the synthesis of 9-(3-deoxy-3-fluoro- α -D-arabinofuranosyl)adenine.

15 Recently the total synthesis of carbohydrates has become the subject of research, and a few papers have appeared. The synthesis requires stereospecific methods, and asymmetric epoxidation and asymmetric aldol reactions have been successfully used. Masamune,
20 Sharpless et al., J. Org. Chem. 47, 1373-81 (1982).

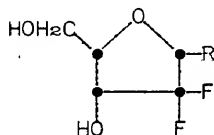
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Summary of the Invention

25 The present invention provides the difluoro-desoxy carbohydrate of the formula

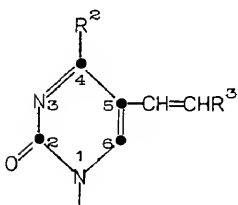
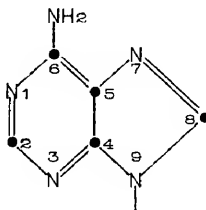
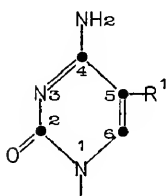
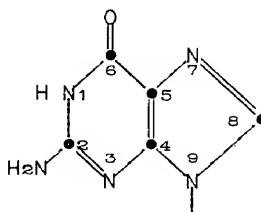
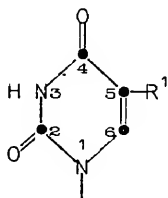


wherein X is hydroxy or a leaving group; and the Y groups independently are hydrogen or hydroxy-protecting groups.

The invention also provides the nucleosides
5 of the formula



wherein R is a base of one of the formulae



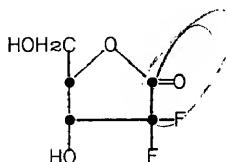
wherein

R^1 is hydrogen, methyl, bromo, fluoro, chloro or iodo;

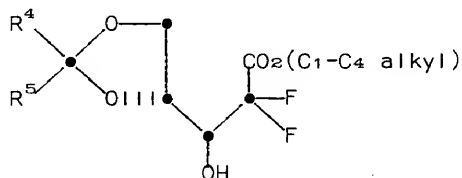
R^2 is hydroxy or amino;

R^3 is hydrogen, bromo, chloro or iodo.

The invention further comprises a process for preparing a lactone of the formula



which process comprises hydrolyzing, under very mild conditions, an alkyl 3-dioxolanyl-2,2-difluoro-3-hydroxypropionate of the formula

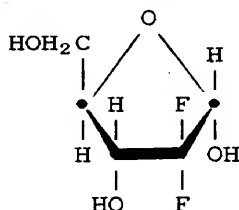


Pharmaceutical compositions comprising a nucleoside of the above formula and a pharmaceutically acceptable carrier, diluent or excipient therefor are provided as yet another aspect of the present invention, as is a method of treating viral infections in mammals employing a present novel compound.

Description of the Preferred Embodiments

All temperatures are described in degrees Celsius in this document. Liquids are reported in volume units.

✓ The structural drawings above do not indicate the stereochemistry of the compounds of the present invention. Compounds of all configurations are believed to be useful, and the stereochemistry of them is accordingly not a limitation. However, it is preferred that the carbohydrate have the configuration of naturally occurring ribose, as follows:



It is further preferred that the configuration of the juncture between the ribose and the base be as follows:



It is believed that pharmaceutical chemists are aware of the bases which are used in the synthesis of the antiviral nucleosides of the present invention,

but the following specific nucleosides are mentioned to assure that every reader understands the type of anti-virals which this invention makes available.

- 5 1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-
2-desoxy-2,2-difluororibose
- 1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-
2,2-difluororibose
- 1-(5-bromo-2,4-dioxo-1H,3H-pyrimidin-1-yl)-
10 2-desoxy-2,2-difluororibose
- 1-(5-chloro-2,4-dioxo-1H,3H-pyrimidin-1-yl)-
2-desoxy-2,2-difluororibose
- 1-(5-iodo-2,4-dioxo-1H,3H-pyrimidin-1-yl)-
2-desoxy-2,2-difluororibose
- 15 1-(4-amino-5-chloro-2-oxo-1H-pyrimidin-1-
yl)-2-desoxy-2,2-difluororibose
- 1-(4-amino-5-bromo-2-oxo-1H-pyrimidin-1-
yl)-2-desoxy-2,2-difluororibose
- 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-
20 desoxy-2,2-difluororibose
- 1-(4-amino-5-iodo-2-oxo-1H-pyrimidin-1-yl)-
2-desoxy-2,2-difluororibose
- 1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-
2-desoxy-2,2-difluororibose
- 25 1-[5-(2-bromovinyl)-4-hydroxy-2-oxo-1H-
pyrimidin-1-yl]-2-desoxy-2,2-difluororibose
- 1-[4-amino-5-(2-bromovinyl)-2-oxo-1H-pyrimidin-
1-yl]-2-desoxy-2,2-difluororibose

- 1-[4-amino-5-(2-iodovinyl)-2-oxo-1H-pyrimidin-1-yl]-2-desoxy-2,2-difluororibose
- 1-[5-(2-chlorovinyl)-4-hydroxy-2-oxo-1H-pyrimidin-1-yl]-2-desoxy-2,2-difluororibose
- 5 1-[4-hydroxy-5-(2-iodovinyl)-2-oxo-1H-pyrimidin-1-yl]-2-desoxy-2,2-difluororibose
- 1-[4-amino-5-(2-chlorovinyl)-2-oxo-1H-pyrimidin-1-yl]-2-desoxy-2,2-difluororibose
- 1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose
- 10 1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose
- 1-(5-fluoro-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
- 15 1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxyllose
- 1-(5-bromo-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxyllose
- 1-(5-chloro-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxyllose
- 20 1-(5-iodo-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxyllose
- 1-(4-amino-5-fluoro-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
- 25 1-(4-amino-5-chloro-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxyllose
- 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxyllose

- 1-(4-amino-5-fluoro-2-oxo-1H-pyrimidin-1-yl)-
2-desoxy-2,2-difluoroxyl
1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-
2-desoxy-2,2-difluoroxyl
5 1-[5-(2-bromovinyl)-4-hydroxy-2-oxo-1H-
pyrimidin-1-yl]-2-desoxy-2,2-difluoroxyl
1-[4-amino-5-(2-bromovinyl)-2-oxo-1H-pyrimidin-
1-yl]-2-desoxy-2,2-difluoroxyl
1-[4-amino-5-(2-iodovinyl)-2-oxo-1H-pyrimidin-
10 1-yl]-2-desoxy-2,2-difluoroxyl
1-[5-(2-chlorovinyl)-4-hydroxy-2-oxo-1H-
pyrimidin-1-yl]-2-desoxy-2,2-difluoroxyl
1-[4-hydroxy-5-(2-iodovinyl)-2-oxo-1H-
pyrimidin-1-yl]-2-desoxy-2,2-difluoroxyl
15 1-[4-amino-5-(2-chlorovinyl)-2-oxo-1H-
pyrimidin-1-yl]-2-desoxy-2,2-difluoroxyl
1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-
desoxy-2,2-difluoroxyl
20 1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-
difluoroxyl

It will be understood that the reactions in
which the novel 2-desoxy-2,2-difluorocarbohydrate of
this invention is coupled with the bases are frequently
25 of a nature such that the hydroxy groups must be pro-
tected to keep them from reacting with the base, or
being decomposed in some manner. The protecting groups
are chosen from the groups used in synthetic organic
chemistry for the purpose. Chemists are accustomed to
30 choosing groups which can be efficiently placed on

hydroxy groups, and which can be easily removed when the desired reaction is complete. Suitable groups are described in standard textbooks, such as Chapter 3 of Protective Groups in Organic Chemistry, McOmie, Ed., Plenum Press, New York (1973); and Chapter 2 of Protective Groups in Organic Synthesis, Greene, John Wiley & Sons, New York (1981).

Typical hydroxy-protecting groups include formyl, 2-chloroacetyl, benzyl, diphenylmethyl, triphenylmethyl, 4-nitrobenzyl, phenoxycarbonyl, t-butyl, methoxymethyl, tetrahydropyranyl, allyl, tetrahydrothienyl, 2-methoxyethoxymethyl, methoxyacetyl, phenoxyacetyl, isobutyryl, ethoxycarbonyl, benzyloxycarbonyl and the like. Silyl hydroxy-protecting groups are often particularly convenient, because most of them are easily cleaved by contact with water or an alcohol. Such groups include especially trimethylsilyl, as well as isopropyl dimethylsilyl, methyl diisopropylsilyl, triisopropylsilyl and the like. The t-butyldimethylsilyl group is a special case and is preferred as the protecting group in this synthesis; it is more difficultly cleaved and requires a reagent such as a hydrohalic acid to remove it from the hydroxy groups.

Ribose or xylose has a hydroxy group at the 1-position of its ring. In order to react the carbohydrate of this invention with the base to form the antiviral compounds of the invention, it is necessary to place a leaving group at the 1-position. The leaving groups used are typical of those used commonly in organic synthesis. The preferred leaving groups are

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3,5-bis(trimethylsilyloxy)-2-desoxy-2,2-
bose

3,5-bis(chloroacetoxy)-2-desoxy-2,2-difluoro-

3,5-bis(2-chlorobenzyloxy)-1-methanesulfonyl-
oxy-2,2-difluororibose

3,5-bis(4-nitrobenzyloxy)-1-(4-toluenesulfonyl-
soxy-2,2-difluororibose

1-(2,4-dibromophenylsulfonyloxy)-3,5-bis(2,2-propionyloxy)-2-desoxy-2,2-difluoroxylse

3,5-bis(benzoyloxy)-1-(o-toluenesulfonyloxy)-

1-bromo-3,5-bis(methoxycarbonyloxy)-1,2-
2-difluoroxylse

3,5-bis(allyloxycarbonyloxy)-1-chloro-1,2-desoxy-2,2-difluoroxylse

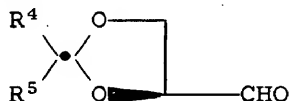
30 3,5-bis(benzyloxycarbonyloxy)-2-desoxy-2,2-
difluoroxylose

- 1-bromo-3,5-bis(4-nitrobenzyloxycarbonyl-
oxy)-1,2-desoxy-2,2-difluoroxyl-ose
- 1-bromo-3,5-bis(tetrahydrothienyloxy)-1,2-
desoxy-2,2-difluororibose
- 5 1-bromo-3,5-bis(isopropyl-dimethylsilyloxy)-
1,2-desoxy-2,2-difluororibose
- 1-(2-chlorophenylsulfonyloxy)-3,5-bis(methoxy-
methoxy)-2-desoxy-2,2-difluororibose
- 3,5-bis(benzyloxymethoxy)-2-desoxy-2,2-di-
10 fluororibose
- 1-(4-nitrophenylsulfonyloxy)-3,5-bis(trityl-
oxy)-2-desoxy-2,2-difluororibose
- 3,5-bis(allyloxy)-1-chloro-1,2-desoxy-2,2-
difluororibose
- 15 2-desoxy-2,2-difluoroxyl-ose
- 3,5-bis(trimethylsilyloxy)-2-desoxy-2,2-
difluoroxyl-ose
- 3,5-dibenzyloxy-2-desoxy-2,2-difluoroxyl-ose
- 3,5-bis(chloroacetoxy)-2-desoxy-2,2-difluoro-
20 xylose
- 3,5-bis(2-chlorobenzyloxy)-1-methanesulfonyl-
oxy-2-desoxy-2,2-difluoroxyl-ose
- 3,5-bis(4-nitrobenzyloxy)-1-(4-toluenesulfonyl-
oxy)-2-desoxy-2,2-difluoroxyl-ose
- 25 1-bromo-3,5-bis(tetrahydrothienyloxy)-1,2-
desoxy-2,2-difluoroxyl-ose
- 1-bromo-3,5-bis(isopropyl-dimethylsilyloxy)-
1,2-desoxy-2,2-difluoroxyl-ose
- 3,5-bis(t-butyldiphenylsilyloxy)-2-desoxy-
30 2,2-difluororibose

- 3,5-bis(formyloxy)-1-isopropylsulfonyloxy-
2-desoxy-2,2-difluororibose
- 3,5-bis(trichloroacetoxy)-1-methanesulfonyl-
oxy-2-desoxy-2,2-difluororibose
- 5 1-chloro-3,5-bis(phenoxyacetoxy)-1,2-desoxy-
2,2-difluororibose
- 1-(2,4-dibromophenylsulfonyloxy)-3,5-bis(2,2-
dimethylpropionyloxy)-2-desoxy-2,2-difluororibose
- 3,5-bis(benzoyloxy)-1-(o-toluenesulfonyl-
oxy)-2-desoxy-2,2-difluororibose
- 10 1-bromo-3,5-bis(methoxycarbonyloxy)-1,2-
desoxy-2,2-difluororibose
- 1-(2-chlorophenylsulfonyloxy)-3,5-bis(methoxy-
methoxy)-2-desoxy-2,2-difluoroxyllose
- 15 3,5-bis(benzyloxymethoxy)-2-desoxy-2,2-di-
fluoroxyllose
- 1-(4-nitrophenylsulfonyloxy)-3,5-bis(trityl-
oxy)-2-desoxy-2,2-difluoroxyllose
- 3,5-bis(allyloxy)-1-chloro-1,2-desoxy-2,2-
20 difluoroxyllose
- 3,5-bis(t-butyldiphenylsilyloxy)-2-desoxy-
2,2-difluoroxyllose
- 3,5-bis(formyloxy)-1-isopropylsulfonyloxy-
2-desoxy-2,2-difluoroxyllose
- 25 3,5-bis(trichloroacetoxy)-1-methanesulfonyl-
oxy-2-desoxy-2,2-difluoroxyllose
- 3,5-bis(allyloxycarbonyloxy)-1-chloro-1,2-
desoxy-2,2-difluororibose
- 3,5-bis(benzyloxycarbonyloxy)-2-desoxy-2,2-
30 difluororibose

10 1-bromo-3,5-bis(4-nitrobenzyloxycarbonyl-
oxy)-1,2-desoxy-2,2-difluororibose

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The carbohydrates are prepared by a process
beginning with the reaction of a D-glyceraldehyde
5 ketonide of the formula



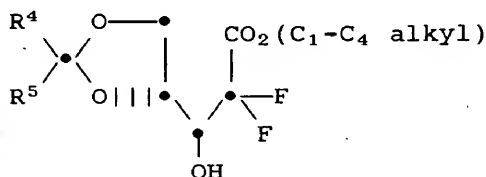
15 wherein R^4 and R^5 are independently C_1 - C_3 alkyl, with
a C_1 - C_4 alkyl bromodifluoroacetate, preferably the
ethyl ester.

15 The preferred glyceraldehyde ketal is the
acetonide wherein R^4 and R^5 are both methyl, which was
first published by Fischer and Baer, Helv. Chim. Acta.
17, 622 (1934). Ethyl bromodifluoroacetate was first
prepared by Morel and Dawans, Tet. 33, 1445 (1977).

20 The reaction of the ketal and the haloacetate is
carried out in the presence of an activated metal such
as magnesium or preferably, zinc. The activation is
most easily obtained by applying ultrasonic energy to
the reaction mixture. Activation by that means com-
25 pensates for the presence of a small amount of water in
the reaction mixture, avoiding the necessity to maintain
anhydrous conditions, and also avoids the necessity to
prepare and carefully store activated metals. However,
the metal may be activated by the customary methods
30 used in the art if desired. Approximately an equimolar
amount of metal is the most advantageous amount.

The reaction has been performed in ethers such as tetrahydrofuran and diethyl ether, at moderate temperatures. However, other organic solvents which are inert to the reaction conditions may be employed, including halogenated alkanes such as chloroform, dichloromethane, trichloroethane and the like, and aromatics including such solvents as benzene, toluene and the xylenes. Temperatures in the range of from about ambient temperature to about 100° are convenient; temperatures from about the ambient temperature to about 80° are preferred. Economically acceptable yields have been obtained in reaction times in the range of from a few minutes to a few hours. It should be noted that the reaction is exothermic, and the mixture may therefore need to be cooled, depending on the scale of the reaction and the rate at which the reactants are added.

The product of the first reaction is an alkyl 3-dioxolanyl-2,2-difluoro-3-hydroxypropionate of the formula

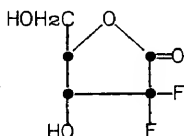


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It appears that the ratio of the 3-R-hydroxy intermediate to its 3-S-hydroxy enantiomer is about 3:1. The 3-R-hydroxy enantiomer has the proper stereochemistry to yield ribose in the natural configuration, and so it is the desired enantiomeric product of the

first step. The 3-R-hydroxy enantiomer can be cleanly separated from the 3-S-enantiomer by standard methods, for example by chromatography on silica gel, eluting with a solvent such as chloroform containing 0.5% methanol (v:v).

The hydroxypropionate, in either form, is hydrolyzed under very mild conditions to form the lactone form of the carbohydrate, of the formula



15 It has been found that proper control of the hydrolysis step will cleave the ketonide function and, unexpectedly, will also cleave the ester group, providing the lactone in a single step. The hydrolysis reagent is preferably a mildly acidic ion exchange resin, of which Dowex 50W-X12 (Dow Chemical Company) is most highly preferred. It is possible to carry out the process with other mild hydrolytic reagents, although it is possible that larger amounts of by-products may be obtained. For example, aqueous acetic acid, or
20 other relatively strong acids such as propionic acid, formic acid, chloroacetic acid, oxalic acid and the like, may be used for the hydrolysis.

25 The hydroxy groups of the lactone should be protected before its keto oxygen is reduced. The usual

reaction conditions are used, depending on the nature of the protecting groups which may be chosen. For example, the t-butyldimethylsilyl group is most conveniently provided in the form of its trifluoromethane-sulfonate, and the protection reaction is carried out in the presence of a base such as lutidine, pyridine and the like. Acyl protecting groups such as acetyl, benzoyl and the like are provided by reacting the lactone with an acylating agent such as an acyl chloride, bromide, cyanide or azide, or with an appropriate anhydride. The reactions are conveniently carried out in a basic solvent such as pyridine, quinoline or isoquinoline, or in a tertiary amine solvent such as triethylamine, tributylamine, methylpiperidine and the like. The reaction may also be carried out in an inert solvent, to which an acid scavenger, such as a tertiary amine, has been added. Acylation catalysts such as 4-dimethylaminopyridine or 4-pyrrolidinopyridine may be used in the reaction, if desired. The acylation reactions which provide protecting groups on the hydroxy groups are carried out at moderate temperatures in the range of from -25° to 100°. Such acylations may also be performed by acid-catalyzed reactions of the appropriate carboxylic acids, in inert organic solvents or neat. Acid catalysts such as sulfuric acid, polyphosphoric acid, methanesulfonic acid and the like are used.

Acyl protecting groups may also be provided by forming an active ester of the appropriate acid, such as the esters formed by such known reagents as dicyclohexylcarbodiimide, acylimidazoles, nitrophenols,

pentachlorophenol, N-hydroxysuccinimide and 1-hydroxy-benzotriazole.

Protecting groups of the ether type are placed by reacting the lactone with, for example, an appropriate diazo compound, such as diazomethane, phenyldiazomethane or a silyldiazomethane. Such reactions are commonly and effectively carried out in solvents including esters such as ethyl acetate, halogenated solvents including dichloromethane and chloroform, and ethers including diethyl ether and tetrahydrofuran. The process is usually carried out at low temperatures from about -50° to about 0°. Such ether-forming reactions may also be carried out with the assistance of reagents such as trimethyloxosulfonium hydroxide, trimethylsulfonium hydroxide and trimethylselenonium hydroxide, in solvents such as dimethylsulfoxide, dimethylformamide, hexamethylphosphoramide, acetone, acetonitrile and the like.

The silyl protecting groups discussed above are placed on the hydroxy groups by the conventional methods, such as by reaction with the appropriate silylcarboxamide or bis(substituted-silyl)carboxamide, or an appropriately substituted silazane. Suitably substituted silyl methanesulfonates, toluenesulfonates and the like are also useful. An equivalent of a base is usually necessary in the reaction mixture, unless a basic solvent such as is discussed above is used as the reaction medium.

When the hydroxy groups have been protected, the keto oxygen of the lactone is reduced to the alcohol, forming the protected 2-desoxy-2,2-difluororibose or xylose of this invention. The most preferred reducing agent is diisobutyl aluminum hydride, used at a low temperature in the range of about -100° to -20°. It is necessary to carry out the reduction very carefully, in order to avoid reducing conditions so vigorous that the ring is opened at the oxygen atom. Other metal hydrides, such as the widely used lithium aluminum hydride, can also be used for the reduction, but it is necessary to keep the temperature quite low and to assure that the hydride is destroyed before the temperature is allowed to rise toward ambient. Accordingly, a solvent with a very low freezing point must be used in the reduction step. Toluene is convenient; other solvents can of course be used, including lower alkanols, especially ethanol, ethers such as diethyl ether, and the like.

An appropriate leaving group must be placed at the 1-position of the carbohydrate, in order to obtain efficient reaction with the base. The preferred leaving group is methanesulfonyl, which is readily provided by reaction with methanesulfonyl chloride in the presence of an equivalent amount of a suitable acid scavenger such as triethylamine and the like. Other sulfonyl leaving groups are provided in the same way by reaction with the appropriate sulfonyl halide.

When a chloro or bromo leaving group is to be used, it is frequently convenient to first make the 1-

acetate derivative, for instance by reaction with acetic anhydride, or another source of acetyl groups, in the presence of an equivalent or more of an acid scavenger. Then the acetate group is displaced with gaseous
5 hydrogen bromide or hydrogen chloride, at a low temperature such as about -50° to about 0° . Since the gaseous hydrogen halide may tend to remove the protecting groups, especially silyl protecting groups, it is necessary to operate this step at quite a low
10 temperature and to add the hydrogen halide slowly in small increments.

The bases used to form the antiviral compounds of the present invention are commonly known to organic chemists, and no discussion of their synthesis
15 is necessary. However, the primary amino groups which are present on some of the bases should be protected before the base is coupled with the carbohydrate. The usual amino-protecting groups are used, including silyl groups such as have been discussed, as well as such
20 typical groups as t-butoxycarbonyl, benzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, formyl, acetyl, and the like.

It is often advisable to convert keto oxygen atoms on the bases to the enol form, in order to make
25 the bases more highly aromatic and thereby allow more ready attack of the base by the carbohydrate. It is most convenient to enolize the oxygens by providing silyl protecting groups for them. The usual silyl protecting groups as discussed above are used for this
30 purpose, also.

The reaction between the protected carbohydrate and the base is preferably carried out neat at an elevated temperature in the range of from about 50° to about 200°. It is possible, however, to use relatively high-boiling solvents for the reaction, such as dimethylformamide, dimethylacetamide, hexamethylphosphoramide and the like. However, if the coupling reaction is carried out under elevated pressure, to avoid distillation of a low-boiling solvent, any convenient inert reaction solvent can be used.

The coupling reaction may be done at low temperatures if a reaction initiator, such as a trifluoromethanesulfonyloxysilane, is used. The usual inert reaction solvents, as discussed above, may be used at temperatures in the range of from about ambient to about 100°.

The final step of the reaction sequence is the removal of the protecting groups. Most silyl protecting groups are easily cleaved by contact with water or an alcohol. The *t*-butyldimethylsilyl protecting group requires acid conditions, such as contact with gaseous hydrogen halide, for its removal.

Acyl protecting groups are removed by simple hydrolysis with strong or moderately strong bases, such as alkali metal hydroxides, at temperatures from about the ambient temperature to about 100°. At least one equivalent of base is needed for each protecting group, of course. Such hydrolyses are conveniently carried out in hydroxylic solvents, especially aqueous alkanols. The reactions may be also carried out, however, in any

convenient solvent, such as polyols including ethylene glycol, ethers such as tetrahydrofuran and the like, ketones such as acetone and methyl ethyl ketone and other polar solvents such as dimethylsulfoxide. The
5 cleavage of acyl protecting groups may also be performed with other bases, including, for example, sodium methoxide, potassium t-butoxide, hydrazine, hydroxylamine, ammonia, alkali metal amides and secondary amines such as diethylamine and the like. The acyl
10 protecting groups can also be removed with acid catalysts, such as methanesulfonic acid, hydrochloric acid, hydrobromic acid, sulfuric acid, or with acidic ion exchange resins. It is preferred to carry out such
15 hydrolyses at a relatively high temperature, such as the reflux temperature of the mixture, but temperatures as low as ambient may be used when particularly strong acids are used.

The removal of protecting groups which are ethers is carried out by known methods, for example,
20 with ethanethiol and aluminum chloride.

None of the reaction steps require unusual excesses of the reactants. As usual in organic syntheses, it is advisable and economical to use a moderate
33 excess, in the range of 1.05X to 2X, for example, of
25 the cheaper reagents to assure that the costlier ones are consumed.

The following preparations and examples further illustrate the synthesis of compounds of the present invention.
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DEChwic.

Preparation 1

ethyl 2,2-difluoro-3-hydroxy-3-(2,2-dimethyl-
dioxolan-4-yl)propionate

5

To 10.2 g. of activated zinc was added a small portion of a solution consisting of 31.8 g. of ethyl bromodifluoroacetate and 22.6 g. of 4-formyl-2,2-dimethyldioxolane in 53 ml. of tetrahydrofuran and 53 ml. of diethyl ether. Care was taken to exclude water from the reaction mixture. The solution began to reflux as soon as the first addition to the activated zinc was made. The remainder of the solution was added dropwise at a rate to maintain gentle reflux throughout the addition time of about 30 minutes. The mixture was then stirred under gentle reflux for 30 minutes more. The reaction mixture was poured into 200 ml. of 1N hydrochloric acid and 200 g. of ice, and the mixture was stirred until all of the ice had melted. The aqueous mixture was then extracted four times with 70 ml. portions of diethyl ether, and the organic layers were combined and washed with 50 ml. of saturated aqueous sodium chloride and with 50 ml. of saturated aqueous sodium bicarbonate, dried over magnesium sulfate and evaporated under vacuum to obtain 26 g. of light yellow oil. The crude product was chromatographed on a 1000 g. silica gel column, eluting with chloroform containing 0.5% methanol to separate the major 3-R-hydroxy product from the minor 3-S-hydroxy product. The ratio of amounts of the two products was about 3:1; the minor product came off the column first.

Evaporation of the fractions containing the 3-R-hydroxy product provided 12.6 g. of the product in substantially pure form. It was identified by mass spectrometry, showing a fragment of weight 239, which agrees with the molecular weight of the desired product less a methyl group which was lost from the acetonide function in the spectrometric measurement. A nuclear magnetic resonance analysis of the 3-R-hydroxy product on a 90 MHz instrument in CDCl_3 showed features at $\delta =$ 3.94-4.45 (m, 5H); 3.14 (d, $J = 4.5\text{Hz}$, 1H); 1.2-1.47 (m, 9H).

Analysis by the same nmr procedure of the 3-S-hydroxy product, of which 4.68 g. was obtained by evaporation of the chromatography fractions containing it, showed features at 3.75-4.47 (m, 6H); 2.95 (d, $J = 8\text{Hz}$, 1H); 1.25-1.5 (m, 9H).

Preparation 2

20 2-desoxy-2,2-difluoro-1-oxoribose

Fifty g. of the 3-R-hydroxy product obtained from a synthesis similar to that of Preparation 1 above was dissolved in 500 ml. of methanol and 250 ml. of water, and 250 g. of Dowex 50W-X12 resin was added. The mixture was stirred at ambient temperature for 4 days, and the mixture was then filtered through a pad of diatomaceous earth filter aid. The filtrate was evaporated to dryness under vacuum to obtain 33.0 g. of the desired product, which was identified by nmr analysis

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on a 90 MHz instrument in CD₃OD: δ = 3.6-4.6 (series
of m, 4H); 4.8 (bs, 2H).

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Preparation 3

5

3,5-bis(t-butyldimethylsilyloxy)-2-desoxy-
2,2-difluoro-1-oxoribose

P
To 13 g. of the product obtained in Prepara-
10 tion 2 above was added 60 ml. of dichloromethane,
22.5 ml. of 2,6-lutidine and 48.2 ml. of trifluoro-
methylsulfonyloxy t-butyldimethylsilane under nitrogen
with mild cooling to keep the temperature below 25°. Within 15 minutes after combining the reagents, the
15 reaction became quite exothermic and the mixture became
thin and easily stirred. The mixture was stirred
overnight. The mixture was diluted with 150 ml. of
ethyl acetate, and was washed successively with 40 ml.
of 1N hydrochloric acid, 40 ml. of saturated aqueous
20 sodium bicarbonate and 40 ml. of saturated aqueous
sodium chloride. It was then dried over magnesium
sulfate and evaporated to dryness under vacuum to
obtain 32.1 g. of crude product, which was chromato-
graphed on 260 g. of 100-mesh silica gel, eluting with
25 10:1 (v:v) chloroform:diethyl ether. The fractions
which contained the desired product were combined and
evaporated under vacuum to obtain 7.8 g. of pure prod-
uct. Other fractions were combined and evaporated to
obtain an additional 10 g. of impure product, which was
30 not further purified. Analysis of the pure product gave

the following results: IR (neat) 1820 cm^{-1} ; nmr
(CDCl_3 , 90 MHz) $\delta = .1-.22$ (m, 12H); $.83-.98$ (m, 18H);
3.63-4.7 (series of m, 4H); mass spec. $m/e = 339 = \text{P}-\underline{\text{t}}$ -
butyl.

5.

Example 1

3,5-bis($\underline{\text{t}}$ -butyldimethylsilyl)-2-desoxy-2,2-
difluororibose

10

A 10.3 g. portion of 3,5-bis($\underline{\text{t}}$ -butyldimethyl-
silyloxy)-2-desoxy-2,2-difluoro-1-oxoribose, obtained
from preparations similar to that of Preparation 3
above, was dissolved in 120 ml. of anhydrous toluene
and cooled to -84° . To the solution was added 26 g.
of diisobutyl aluminum hydride, added over a period of
20 minutes with constant stirring. The reaction mixture
was held below -65° at all times. Two hours after the
first addition of hydride, the reaction mixture was
quenched with methanol at -20° , additional cold methanol
was added until no more gassing occurred. The mixture
was then allowed to warm slowly to ambient temperature,
and was washed with 100 ml. of 0.1N hydrochloric acid.
The aqueous layer was then washed with 100 ml. of
diethyl ether, and then three times with 50 ml. portions
of diethyl ether. The organic layers were combined,
washed with 100 ml. of saturated aqueous sodium bi-
carbonate, dried over magnesium sulfate and evaporated
under vacuum to dryness to obtain 8.2 g. of the desired
product in crude form.

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This material may be chromatographed, if necessary, on silica gel (25 g. silica/1 g. of crude product) using 100% dichloromethane for elution. nmr (CDCl₃, 90 MHz) δ = 1.1-1.24 (m, 12H); 1.85-1.0 (m, 18H); 3.33-4.63 (series of m, 5H); 5.0-5.27 (dd, 1H); mass spec. m/e = 341 = P-t-butyl; $[\alpha]_D^{25} = +25.1^\circ$.

Example 2

10 3,5-bis(t-butyltrimethylsilyloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluororibose

p
An 0.5 g. portion of 3,5-bis(t-butyltrimethylsilyloxy)-2-desoxy-2,2-difluororibose was dissolved in 15 5 ml. of anhydrous dichloromethane and 0.17 g. of triethylamine. To the solution was added, with mild cooling, 0.11 ml. of methanesulfonyl chloride. After three hours of stirring under nitrogen at about 25°, the mixture was evaporated under vacuum, and the residue 20 was taken up in 10 ml. of ethyl acetate. The solution was extracted with 3 ml. of saturated aqueous sodium bicarbonate, and then successively with 3 ml. of 1N hydrochloric acid, 3 ml. of water and 3 ml. of saturated aqueous sodium chloride. The organic solution was then 25 dried over sodium sulfate and concentrated under vacuum to obtain 0.59 g. of the desired product, nmr (CDCl₃, 90 MHz) δ .05-.16 (m, 12H); .78-.90 (m, 18H); 3.0 (s, 3H); 3.63-4.59 (series of m, 4H); 5.67-5.9 (dd, 1H); mass spec. m/e = 419 = P-t-butyl.

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30

Example 3

1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-
2-desoxy-2,2-difluororibose

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To 2.59 g. of 3,5-bis(t-butyldimethylsiloxy)-
1-methanesulfonyloxy-2-desoxy-2,2-difluororibose was
added 1.60 g. of 5-methyl-2,4-bis(trimethylsilyloxy)-
pyrimidine and 45 ml. of dry 1,2-dichloroethane. To
10 this mixture was added 1.45 g. of trifluoromethane-
sulfonyloxytrimethylsilane, and the clear solution was
stirred under nitrogen at reflux for about 2-3 hours.
The reaction was then cooled to ambient temperature and
1.35 ml. of methanol were added and the suspension was
15 stirred for 30 minutes. The precipitate was filtered and
the filtrate was reduced to one-half its volume under
vacuum and then diluted with an equal volume of dichloro-
methane. The solution was washed with saturated aqueous
sodium bicarbonate and then with saturated aqueous
20 sodium chloride, and dried over anhydrous sodium sulfate.
The solution was filtered and the filtrate was saturated
with anhydrous hydrogen bromide. The reaction mixture
was stirred for 30 minutes and was then concentrated
under vacuum. The residue was dissolved in methanol and
25 the solution was evaporated to dryness under vacuum.
The residue was dissolved in water and the solution was
extracted twice with diethyl ether. The water layer was
then evaporated to dryness. The residue was taken up in
ethanol and evaporated repeatedly to azeotrope off all
30 water. One g. of crude product was obtained, and was
chromatographed on 30 g. of Woelm silica gel (70-150 mesh),

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eluting with ethyl acetate to yield 0.76 g. of desired product. It was further purified by recrystallization from ethyl acetate to obtain 0.37 g. of white crystalline product. nmr (CD₃OD, 90 MHz) δ 1.93 (s, 3H); 3.5-4.67 (series of m, 4H); 4.83 (bs, 3H); 6.3 (t, J = 9Hz, 1H); 7.47 (m, 1H); mass spec. m/e = 278 = Parent.

Close
Example 4

10 1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-
2-desoxy-2,2-difluororibose

p
15 A 5.4 g. portion of 3,5-bis(t-butyldimethyl-
silyloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluoro-
ribose and 5.4 g. of 5-methyl-2,4-bis(trimethylsilyl-
oxy)pyrimidine were combined and heated under nitrogen
with stirring at 100° for one hour, and then at 150°
for one hour. The mixture was then cooled to ambient
temperature and diluted with 25 ml. of water and 10 ml.
20 of methanol. The slurry was filtered over a diatomaceous
earth filter pad, and the cake was washed with acetone.
The combined filtrate was evaporated under vacuum to
obtain 5.3 g. of an oily residue. The residue was
dissolved in 10 ml. of acetone and loaded on a 4.5 cm.
25 column packed with 80 g. of silica gel. It was eluted
with 15:1:1 dichloromethane:methanol:triethylamine.

The first 100 ml. of eluent was discarded, and the next 300 ml. was evaporated under vacuum to obtain 4.1 g. of syrupy crude product, which was dissolved in 40 ml. of acetone. Hydrogen chloride was
5 bubbled through the solution for 1 hour, and then hydrogen bromide was bubbled through for 1 hour more. The solution was then evaporated at 62° to obtain 4.4 g. of oily dark product.

The above product was dissolved in 10 ml. of
10 warm 3:1 dichloromethane:acetic acid, and loaded on a 4.5 cm. column packed with 45 g. of silica gel. The eluent used for the first 1000 ml. was 3:1 dichloromethane:acetic acid, and thereafter it was acetic acid alone. Most of the desired product was in the fractions
15 between 1000 and 1400 ml. off the column, as determined by thin layer chromatography on silica gel using 15:1 dichloromethane:methanol. Those fractions were combined and evaporated under vacuum, and the residue was taken up in 15 ml. of cold acetone and filtered. The
20 filtrate was stripped under vacuum to obtain an oil, which was dissolved in 5 ml. of acetone and chromatographed over 20 g. of silica gel with 15:1 dichloromethane:methanol. The product-containing fractions were combined and evaporated under vacuum to obtain
25 300 mg. of a semi-solid. That product was taken up in 5 ml. of acetone and filtered, and the filtrate was evaporated under vacuum to obtain 230 mg. of light brown semi-solid. It was dissolved in 10 ml. of saturated aqueous sodium bicarbonate, and the solution
30 was extracted twice with 15 ml. portions of diethyl

ether. The aqueous phase was then evaporated under vacuum, the residue was slurried in acetone and filtered, and the filtrate was evaporated under vacuum to obtain 140 mg. of the desired product as a tan viscous oil.

The following example illustrates a preferred method of isolating a compound of the present invention.

Example 5

1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-
2-desoxy-2,2-difluororibose

To 80.0 g. of 3,5-bis(t-butyldimethylsilyloxy)-
1-methanesulfonyloxy-2-desoxy-2,2-difluororibose under a nitrogen atmosphere was added 1.4 l. of freshly distilled methylene chloride and 49.5 g. of 5-methyl-2,4-bis-(trimethylsilyloxy)pyrimidine. To this mixture was added 44.8 g. of trifluoromethanesulfonyloxytrimethylsilane and the reaction mixture was refluxed for approximately 3½ hours. The reaction mixture was stirred at room temperature overnight and 41.6 ml. of methanol was added thereto. The resulting mixture was stirred for approximately 30 minutes and the precipitated solid was collected by filtration. The filtrate was concentrated under vacuum at 45° to provide a dark oil which was dissolved in 500 ml. of methylene chloride saturated with anhydrous hydrogen bromide. The resulting suspension was stirred for approximately 3 hours after which the volatiles were removed under vacuum at 45°. The residue was dissolved in 100 ml. of 10% sodium

bicarbonate and 100 ml of diethyl ether. The aqueous layer was separated and concentrated in vacuo at 50° to provide a residue which was triturated three times with 100 ml. portions of hot ethyl acetate. The organic layers were combined and evaporated under vacuum at 45° to provide a residue which was dissolved in 50 ml. of water. This solution was chromatographed in 10 ml. portions on a Waters Prep 500 C¹⁸ reverse phase column using water/methanol (v:v, 9:1) as the eluent to provide 2.21 g. of 1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose. nmr (CD₃OD, 90 MHz) δ 1.9 (s, 3H), 3.65-4.65 (m, 4H), 4.83 (s, 3H), 6.12 (dd, J=7Hz, 12Hz, 1H), 7.70 (s, 1H); mass spec. m/e = 278 = P.

Example 6

1-(5-iodo-2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

To a solution of 1.99 g. of 3,5-bis(t-butyl-dimethylsilyloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluororibose in 35 ml. of dry methylene chloride under a nitrogen atmosphere was added 2.08 g. of tris (tri-methylsilyl)-5-iodocytosine and 1.11 g. of trifluoromethanesulfonyloxytrimethylsilane. The reaction mixture was refluxed for approximately 16 hours and cooled to room temperature. Five milliliters of methanol were added to the mixture which was stirred for approximately 30 minutes. The precipitated solid was collected by filtration and the filtrate was evaporated to dryness

under vacuum. The residue was dissolved in 20 ml. of methylene chloride saturated with anhydrous hydrogen bromide to provide a suspension which was stirred for approximately 3 hours at room temperature. The volatiles were evaporated under reduced pressure at 45° and the resulting residue was dissolved in 15 ml. of water. The solution was neutralized to a pH of approximately 7 to 8 with 10% sodium bicarbonate and washed one time with 10 ml. of ethyl acetate. The aqueous layer was chromatographed on a Whatman Prep ODS-3 reverse phase column in 2 ml. portions employing water/methanol (v:v, 9:1) as the eluent to provide 30 mg. of 1-(5-iodo-2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose, nmr (CD₃OD, 90 MHz), δ 3.47-4.66 (m, 4H), 4.78 (s, 4H), 6.14 (2e, J=7Hz, 1H), 8.32 (s, 1H); mass spec. m/e = 389 = p.

Example 7

1-(5-fluoro-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

A solution of 1.1 g. of 3,5-bis(t-butyl-dimethylsilyloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluororibose, 0.83 g. of 5-fluoro-2,4-bis(trimethylsilyloxy)pyrimidine, and 0.655 g. of trifluoromethanesulfonyloxytrimethylsilane in 20 ml. of methylene chloride was refluxed for approximately 17 hours under nitrogen. The reaction mixture was cooled to room temperature and 3 ml. of methanol was added thereto. The resulting solution was stirred for approximately 30

minutes at room temperature and the precipitated solid was collected by filtration. The filtrate was evaporated to dryness under vacuum at 50° and the residue was dissolved in 15 ml. of methylene chloride saturated with anhydrous hydrogen bromide. The resulting suspension was stirred for approximately 30 minutes and the volatiles were removed in vacuo at 45°. The residue was dissolved in 15 ml. of water and the aqueous solution was extracted one time with 10 ml. of ethyl acetate. The aqueous layer was neutralized with sodium carbonate to a pH of approximately 7 and the solution was evaporated to dryness in vacuo at 50°. The residue was dissolved in about 6 ml. of water and the resulting solution was chromatographed in 2 ml. portions on a Whatman ODS-3 50cm reverse-phase column using water as the eluent to provide 30 mg. of 1-(5-fluoro-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose. nmr (CD₃OD, 90 MHz) δ 3.63-4.6 (m, 4H), 4.79 (s, 3H), 6.04 (t, J=7 Hz, 1H), 8.07 (d, J=6 Hz, 1H); mass spec. m/e = 282 = p.

Example 8

1-(2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

Under nitrogen, to a solution of 47.3 g. of 3,5-bis(t-butyldimethylsilyloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluororibose in 940 ml. of methylene chloride was added 48 g. of bis(trimethylsilyl)-N-acetylcytosine. To this mixture was added 39.23 g. of

trifluoromethanesulfonyloxytrimethylsilane and the resulting mixture was stirred under reflux for approximately 15 hours. The reaction mixture was cooled to room temperature and 16 ml. of methanol was added thereto. The resulting solution was stirred for approximately 30 minutes and concentrated to about one-half of its original volume. The solution was cooled in ice and the precipitated solid was collected by filtration. The filtrate was shaken one time with approximately 300 ml. of 10% sodium bicarbonate and one time with 100 ml. of brine. The organic layer was evaporated to dryness in vacuo at 45° and the residue was dissolved in 1.3 l. of methanol saturated with ammonia. The resulting suspension was allowed to stir overnight at room temperature and the volatiles were removed under vacuum at 45°. The residue was dissolved in 275 ml. of methanol and 100 g. of Bio Rad ion exchange resin (AG 50WX8) was added thereto. The suspension was stirred at room temperature overnight and the resin was collected by filtration. The resin was rinsed with 100 ml. of methanol and suspended in 100 ml. methanol and 50 ml. of concentrated ammonium hydroxide. The resin containing suspension was stirred vigorously for 15 minutes and the resin was collected by filtration. This procedure was twice repeated with additional ammonia saturated methanol. The basic methanolic filtrates were combined and evaporated at 45° under vacuum to provide 13.8 g. of a solid. This material was chromatographed on a Waters Prep 500 C¹⁸ Reverse Phase Column with water as the eluent to provide 1.26 g. of 1-(2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-

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2,2-difluororibose. nmr (CD₃OD, 90 MHz) δ 3.7-4.65 (m, 4H), 4.83 (s, 4H), 5.97 (d, J=8 Hz, 1H), 6.24 (t, J=7 Hz, 1H), 7.88 (d, J=8 Hz, 1H); mass spec. m/e = 263 = p.

5

Example 9

1-(2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylse

P
10 Under nitrogen, to 23 g. of 3,5-bis(t-butyl-dimethylsilyloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluoroxylse was added 23 g. of tris(trimethylsilyl)-cytosine and 300 ml. of methylene chloride. To this mixture was added 10.84 g. of trifluoromethanesulfonyl-
15 oxytrimethylsilane and the mixture was refluxed for approximately 16 hours. The mixture was cooled to room temperature and 20 ml. of methanol was added thereto. The solution was stirred vigorously for approximately 1 hour at room temperature and the pre-
20 cipitated solid was collected by filtration. One hundred milliliters of water were added to the organic layer and the suspension was stirred vigorously for 30 minutes. The organic layer was separated and evaporated to dryness under reduced pressure to provide 11.2 g. of
25 a brown oil. The residue was dissolved in 97 ml. of methanol to which 33 g. of Bio Rad AG 50X8 cationic exchange resin was added. The suspension was stirred for approximately 16 hours at room temperature and the resin was collected by filtration. The resin was washed
30 with 50 ml. of methanol and stirred vigorously in a solution of 100 ml. of methanol and 100 ml. of ammonium

hydroxide. This procedure was twice repeated and the combined filtrates were concentrated under vacuum at 50° to provide 2.09 g. of a yellow residue. The residue was suspended in 25 ml. of water and stirred vigorously for 15 minutes. The insoluble precipitate was collected by filtration to provide 0.25 g. of a substance labelled Compound A. The filtrate was concentrated in vacuo at 50° to provide 0.86 g. of a compound labelled B. Compound A was dissolved in 20 ml. of methanol and stirred for 3 days with Bio Rad AG 50WX8 at ambient temperature. The resin was filtered and slurried in 30 ml. of a 1:1, v:v solution of methanol/-ammonium hydroxide. The resin was again filtered and the filtrate was concentrated in vacuo at 50° to provide 0.14 g. of 1-(2-deoxy-2,2-difluoro- β -D-xylofuranosyl)-cytosine. nmr (CD₃OD, 90 MHz) δ 3.72-4.34 (m, 4H), 4.78 (s, 4H), 5.86 (d, J=8 Hz, 1H), 6.17 (d, J=15 Hz, 1H), 7.78 (d, J=8 Hz, 1H); mass spec. m/e = 263 = p.

The compound labelled B was chromatographed on a Whatman 50cm ODS-3 Reverse Phase Prep column using water/methanol (v:v, 1:1) as the eluent to provide 0.06 g. of 1-(2-deoxy-2,2-difluoro- α -D-xylofuranosyl)-cytosine. nmr (CD₃OD, 90 MHz) δ 3.53-3.9 (m, 2H), 4.1-4.57 (m, 2H) 4.83 (s, 4H), 5.9 (d, J=8 Hz, 1H), 6.3 (dd, J=7 Hz, 12Hz, 1H) 7.55 (d, J=8 Hz, 1H); mass spec. m/e = 263 = p.

Clue
Ca
p

Example 10

1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

- 5
- A solution of 0.19 g. of 1-(2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose in 16 ml. of glacial acetic acid and 4 ml. of water was refluxed for approximately 24 hours. The reaction mixture was
- 10 cooled to ambient temperature and the volatiles were removed under vacuum at a temperature in the range of about 60° to about 70°. The residue was evaporated several times with 5 ml. of toluene. The residue was dissolved in 12 ml. of methanol and the resulting
- 15 solution was cooled in a salt/ice bath to approximately -15°. The solution was saturated with anhydrous ammonia and allowed to stir overnight at room temperature. The volatiles were evaporated under reduced pressure at 45° and the residue was suspended in 5 ml. of hot water.
- 20 The insoluble material was collected by filtration and the filtrate was chromatographed on a Whatman 50cm Partisil ODS-3 Reverse Phase column using water/methanol (v:v, 9:1) as the eluent to provide 0.05 g. of the product containing a small trace of unreacted starting
- 25 material. This unreacted starting material was removed by passing a solution of the solid in approximately 5 ml. of methylene chloride containing 10% methanol by volume through a Waters Silica Sep-Pak. The eluent was evaporated in vacuo at 45° to provide 0.036 g. of
- 30 1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose. nmr (CD₃OD, 90 MHz) δ 3.54-4.48 (m, 4H),
- 67, 14

4.83 (s, 3H), 5.69 (d, J=8 Hz, 1H), 6.10 (dd, J=7 Hz, 9Hz, 1H), 7.8 (d, J=8 Hz, 1H); mass spec. m/e = 264 = p.

Example 11

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1-(5-methyl-2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

Under nitrogen, a solution of 1.86 g. of 3,5-bis(t-butyldimethylsilyloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluororibose, 1.87 g. of tris(trimethylsilyl) cytosine and 1.34 g. of trifluoromethanesulfonyloxytrimethylsilane in 37 ml. of dry methylene chloride was refluxed overnight. The reaction mixture was cooled to room temperature and 1 ml. of methanol was added thereto. The precipitated solid was collected by filtration and the filtrate was evaporated under vacuum at 45°. The residue was dissolved in approximately 20 ml. of water and this solution was concentrated to approximately one-half of its original volume by evaporation under vacuum at 50°. The precipitate that formed was collected by vacuum filtration and the filtrate was evaporated in vacuo at 50°. The residue was triturated several times with 10 ml. portions of warm acetone. The organic extracts were combined and evaporated in vacuo at 45° to provide 1.67 g. of a yellow oil. This material was dissolved in 15 ml. of methanol/water (v:v, 2:1) and stirred overnight with 5 g. of Bio Rad AG 50WX8. The suspension was saturated with anhydrous ammonia and stirred for approximately 10 minutes. The resin was collected by vacuum filtration and suspended

in 30 ml. of methanol/ammonia (1:1, v:v). The suspension was stirred for approximately 10 minutes. The resin was collected by filtration and the basic filtrates were combined and concentrated under vacuum at 50° to provide 1.5 g. of an orange oil. The oil was dissolved in 10 ml. of water and this solution was chromatographed in 2 ml. portions on a Whatman Partisil ODS-3 50cm Reverse Phase Prep column using water as the eluent to provide 0.07 g. of 1-(5-methyl-2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose. nmr (CD₃OD, 90 MHz) δ 1.94 (s, 3H), 3.53-4.62 (m, 4H), 4.75 (s, 4H), 6.17 (t, J=8 Hz, 1H), 7.67 (s, 1H); mass spec. m/e = 277 = p.

In addition to the antiviral utility of the present compounds, certain of the compounds of the present invention have also demonstrated excellent oncolytic activity in standard cancer screens. A particularly preferred compound with this utility is the compound of Example 8, 1-(2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose. This compound has demonstrated activity in tumor systems L1210V lymphocytic leukemia, 6C3HED lymphosarcoma, CA-755 adenocarcinoma, P1534J lymphatic leukemia and X5563 plasma cell myeloma. When used for cancer chemotherapy, dosages per day of the active compounds will be in the range of about 0.1 to about 1200 mg./kg. of body weight. In the treatment of adult humans, the range of about 0.1 to about 50 mg./kg., in single or divided doses, is preferred.

The antiviral effect of the compounds of this invention has been shown by a proven in vitro test, which was carried out as follows. A representative compound, that of Examples 3 and 4 above, was tested, and is referred to in the following description by the code term "DFAT".

Test 1

African green monkey kidney cells (BSC-1) were grown in 25 cm.² Falcon flasks at 37° in medium 199 with 5 percent inactivated fetal bovine serum (FBS), penicillin (150 units/ml.) and streptomycin (150 mcg./ml.). When confluent monolayers were formed, the supernatant growth medium was removed and 0.3 ml. of an appropriate dilution of pseudorabies virus or Herpes simplex virus, type I, was added to each flask. After adsorption for one hour at room temperature, the virus infected cell sheet was overlaid with a medium comprising one part 1 percent Ionagar No. 2 and one part double strength medium 199 with FCS (fetal calf serum), penicillin, and streptomycin and also containing crude DFAT at concentrations ranging from 100 to 0.39 micrograms per milliliter (mcg./ml.). A flask containing no DFAT served as a control. The stock solution of DFAT was made up in dimethylsulfoxide at a concentration of 104 mcg./ml. The flasks were incubated for 72 hours at 37°. Plaques were seen in those areas where the virus infected and reproduced in the cells. A solution of 10 percent formalin and 2 percent sodium acetate was added to each flask to inactivate the virus and fix the

cell sheet to the surface of the flask. The virus
plaques, irrespective of size, were counted after
staining the surrounding cell areas with crystal
violet. The plaque count was compared to the control
5 count at each drug concentration. The activity of the
compound was expressed as percentage plaque inhibition.

The results of these evaluations are reported
below in Tables 1 and 2.

T0440X

TABLE 1

Percent Plaque Inhibition at Specified Mcg./Ml. of
DFAT Concentration in Agar Overlay

Herpes simplex, type I

	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39
	96%	72%	53%	35%	15%	12%	0	8%	4%

T0450X

Table 2

Percent Plaque Inhibition at Specified
Mcg./Ml. of DFAT Concentration in Agar Overlay

	Pseudorabies Virus				
	100	50	25	12	6
DFAT	23%	18%	15%	12%	6%
					3
					5%

The antiviral nucleosides of the present invention are used for the treatment of viral infections in the manner usual in the treatment of such pathologies. The compounds are effective for the treatment of viral infections in general, and most particularly in the treatment of infections caused by viruses of the herpes genus.

The compounds are effectively administered orally, topically or parenterally. In general, dosage rates in the range of from about 5 mg./kg. to about 500 mg./kg. are useful. It is more preferred to administer rates in the range of from about 10 mg./kg. to about 100 mg./kg.

The compounds are usually used in medicine in the form of one of the pharmaceutical compositions of the present invention, which compositions are novel and important because of the presence of the novel nucleosides in them. The formulation of the compositions is conventional, and follows the usual practices of pharmaceutical chemists. When a nucleoside of the present invention is to be administered topically, it is formulated as a topical composition, such as a cream or ointment to be rubbed into the affected tissue. Creams are emulsions of an oily phase and an aqueous phase, in which the nucleoside is dissolved or suspended. Ointments are greasy or waxy compositions, in which the nucleoside may be soluble but may be suspended, if it is insoluble at the desired concentration.

Parenteral compositions are preferably formulated in such a way that the nucleoside can be dissolved for injection, but most of the nucleosides are by no means highly water-soluble. Thus, it is more
5 common for a parenteral product to be formulated as a dried powder of the nucleoside and physiologically-acceptable suspending agents, such as starch, sugar and the like, to which sterilized water is added to form a suspension to be injected. Parenteral compositions can
10 be formulated in aqueous bases containing moderate amounts of physiologically-acceptable solvents, such as propylene glycol and the like, and such compositions may be capable of dissolving the present nucleosides at acceptable concentrations.

15 A great many types of orally administered compositions are in common use, including unit dosage forms such as tablets and capsules, and liquid dosage forms such as suspensions. In general, unit dosage forms are preferred in pharmacy and are formulated in
20 such a way as to provide the usual dose in one or a small number of tablets or capsules. The formulation of tablets, making use of appropriate lubricants, binding agents and disintegration agents, is and long has been thoroughly understood by pharmaceutical chem-
25 ists. The formulation of capsules involves only the dilution of the nucleoside with an appropriate proportion of an inert powdery substance, such as lactose, to provide the proper bulk to fill the desired size of capsule. The formulation of orally-administered
30 suspensions is carried out by finely grinding the

nucleoside, and intimately mixing it with a comparatively viscous aqueous-base liquid. The viscosity is adjusted by the addition of pharmaceutically-acceptable thickening or gel-forming agents including vegetable
5 gums, chemically-modified cellulose derivatives and the like. Of course, appropriate flavors are used to make the suspensions organoleptically acceptable.